The efficacy of a highly concentrated fluoride dentifrice on bovine enamel subjected to erosion and abrasion

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Abstract: BACKGROUND: Researchers have proposed the use of fluoride for the prevention of enamel wear; however, only limited information is available about the impact of fluoridated dentifrices. Because tooth wear is a well-recognized dental problem, the authors conducted an in situ, ex vivo study to assess the efficacy of a highly concentrated fluoride dentifrice on bovine enamel subjected to erosion and abrasion. METHODS: The authors conducted a double-blind, crossover in situ study consisting of three phases (seven days each). In each phase, the authors tested one of the dentifrices (5,000 parts per million fluoride [F]; 1,100 ppm F; no F). They performed erosive challenges with the use of cola drink (60 seconds, four times per day) and abrasive challenges via toothbrushing (30 seconds, four times per day). The authors determined the enamel loss via profilometry. RESULTS: The authors tested the data by using two-way analysis of variance ($P < .05$). For the erosion-plus-abrasion condition, the study results showed that enamel wear was significantly higher than that with erosion alone. The findings showed no significant differences between the dentifrices regarding enamel wear. CONCLUSIONS: Within the in situ, ex vivo conditions of this study, the authors concluded that the highly concentrated fluoride dentifrice did not have a protective effect on enamel against erosion and erosion plus toothbrushing abrasion. CLINICAL IMPLICATIONS: Patients at risk of developing enamel erosion should benefit from preventive measures other than fluoride dentifrice, because even a highly concentrated fluoride dentifrice does not appear to prevent enamel erosion.

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Research—Full articles; Advances in Dental Products

Background. Fluoride has been proposed for the prevention of dental wear, however only limited information is available about the impact of fluoridated dentifrices.

Aim: As tooth wear is a well recognized dental problem, the aim of this *in situ*/*ex vivo* study was to assess the efficacy of a highly concentrated fluoride dentifrice on enamel subjected to erosion and abrasion.

Methods. A crossover and double-blind *in situ* study of 3 phases (7d) was conducted. In each phase one of the dentifrices (5,000 ppm F; 1,100 ppm F; no F) were tested. Erosive challenges were performed by Cola drink (60s, 4x/day) and the abrasive by toothbrushing (30s, 4x/day). The enamel loss was determined by profilometry.

Results. Data were tested using 2-way ANOVA ($p<0.05$). For the condition erosion plus abrasion, the wear was significantly higher compared to erosion alone. There were no significant differences among the dentifrices regarding enamel wear.

Conclusions. Under the chosen *in situ*/*ex vivo* conditions, it is concluded that the highly concentrated fluoride dentifrice did not have a protective effect on enamel against erosion and erosion plus toothbrushing abrasion.

Clinical Implications. Risk patients for erosion should benefit from other preventive measures in addition to fluoride dentifrice, since even a highly concentrated fluoride dentifrice is not suitable for enamel erosion prevention.

Introduction

Pathological tooth wear is a well recognized dental problem in clinical dental practice[1]. A major factor in tooth wear is the interaction between erosion of dental hard tissues by dietary or endogenous acids and intra-oral abrasive forces, such as toothbrushing[2]. A number of studies have shown that acidic fluids cause both loss and softening of the enamel[3-6]. As consequence, the softened enamel surface is more susceptible to abrasion, which might increase the wear of eroded dental hard tissues[3,4,6,7]. As these erosive and abrasive processes are frequently observed, efforts have been made to elucidate how erosive/abrasive lesions could be prevented.

The literature shows that saliva and fluoridation measures are the most important factors in the repair of eroded enamel[7,8], since calcium phosphate minerals and calcium fluoride precipitate from saliva and fluoride application respectively, thus rendering eroded tooth surfaces more resistant to brushing abrasion[9]. While several studies showed that the application of highly concentrated fluoride gels might be effective in reducing erosive mineral loss[10,11] and increasing abrasion resistance[8,10], only limited information is available about the impact of fluoridated dentifrices. While some studies showed a limited beneficial effect of commercial fluoridated toothpastes on erosion and abrasion[12-14], other studies did not[9,15,].

The calcium-fluoride-like material deposited from topical fluoride application has been associated with the beneficial effect of fluoride against erosive/abrasive lesions. The thickness of this calcium-fluoride-like layer might be increased by the application of higher concentrated fluoride agents[16]. Thus, it would be interesting to analyze if a highly concentrated fluoride dentifrice (5,000 ppm F) might achieve higher preventive effect against erosion and erosion plus abrasion. Therefore, the aim of this study was to assess the effect of a highly concentrated fluoride dentifrice (5,000 ppm F) on enamel subjected to erosion or to erosion plus abrasion using an *in situ*/*ex vivo* protocol.

Material and Methods

Experimental design

This study was approved by the Research and Ethics Committee of the Bauru School of Dentistry, University of São Paulo (Proc n^{o} 104/2006). It involved a crossover and double blind design performed in three phases of 7 days each, with a washout period

of 7 days between the phases. Sample size calculation was based on a previous study[14] and designed to have a statistical power of 75% with an alpha of 5%. Ten adult volunteers with a mean age of 24 years (range 19-30 yr), with good oral health, and residing in a fluoridated area (0.70 mg F/L) took part in this study. They wore acrylic palatal appliances, which contained 4 bovine enamel slabs divided in two rows: erosion (1) and erosion plus abrasion (2). The use of two conditions in the same intraoral palatal appliance was supported by the absence of a cross-effect in previous studies[14,17]. The tested dentifrices were: Duraphat®-D (5,000 ppm F, NaF, silica, RDA 77±11, pH 8.0); Crest®-C (1,100 ppm F, NaF, silica, RDA 100, pH 7.0) and placebo Duraphat®-P (no F, silica, pH 8.0). The RDA of Duraphat® was checked at Zurich University, according to Barbakow et al. (1989)[18] and the RDA of the placebo is expected to be similar to Duraphat®, since they have the same formulation. The RDA of Crest was reported by Rice et al. (2001)[19]. The erosion was performed with cola drink (pH 2.6, 60 s) and the abrasion by toothbrushing with the respective dentifrice slurry (30 s), 4 times a day. After each phase, enamel loss was determined by profilometry.

Enamel slabs and palatal appliance preparation

One hundred and twelve enamel slabs (4x4 mm) were prepared from extracted bovine incisors, which were sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. The enamel surface of the slabs was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and polished with diamond spray (1 µm; Buehler). For allocation of the samples to the groups, the surface microhardness was determined by performing five indentations in different regions of the slabs (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). In order to maintain reference surfaces for lesion depth determination by profilometry, two layers of nail varnish were applied on half of the surface of each slab. Two slabs were fixed with wax into two cavities (5x5x3 mm) located at each left and right sides of the intraoral palatal appliances.

Treatments

Seven days prior to the beginning and throughout the experimental phase (7 d), the volunteers brushed their teeth with one of the respective dentifrices. In this crossover protocol, the volunteers were randomly allocated to the treatments and participated in 3 phases. In the first 12 hours of each intraoral phase, the slabs were not subjected to erosive and abrasive treatment to allow the formation of a salivary pellicle[7,14]. According to

Amaechi et al.[20] one hour pellicle already exhibits a protective effect against erosion. On the following 7 days, erosive and abrasive challenges were made extraorally 4 times a day at predetermined times (8.00, 12.00, 16.00 and 20.00 h) after the meals[7,14].

For erosion of the enamel slabs, the volunteers were instructed to remove the appliance and immerse it in a cup containing 150 mL of a freshly opened bottle of regular Coke® (Coca-cola Company, pH 2.6, Spal, Porto Real, RJ, Brazil) at room temperature for 1 minute. During this *ex vivo* erosion, the volunteers brushed their teeth with one of the respective dentifrices using a soft end-rounded toothbrush (Sorriso Infantil®, Brazil) with a small portion of the dentifrice (approximately 0.3 g). After erosion of the slabs, 1 drop (around 35 µL) of the dentifrice slurry was dripped on the enamel surface of each slab. While no treatment was performed in one row (1), the other row (2) was brushed using a soft end-rounded electric toothbrush (Colgate® Montions Multi-action, Brazil) for 30 s (166 oscillations/s) *ex vivo*. Volunteers were trained and instructed to perform this procedure carefully and to avoid a carry-across effect of the treatments. The appliances were replaced into the mouth and the volunteers rinsed with water (10 mL, 5 s).

The dentifrice slurry was prepared with the respective dentifrice and de-ionized water in the proportion 1:3 (g/mL) by the researchers. All solutions and dentifrices used by the volunteers were placed in separated vials which did not allow their identification in order to conform to the blind protocol of the study. The volunteers received instructions to wear the appliances continuously for 24 h but to remove them during meals (4 times a day, 1 h each). In this period the appliance was stored in wet gauze. The volunteers received oral and written information to refrain from using any fluoridated product.

Enamel loss assessment

After 7 days, the enamel slabs were removed from the appliances and the nail varnish on the reference surfaces was removed carefully with acetone-soaked cotton wool[6]. Surface profiles of the enamel samples were obtained with a stylus profilometer (Mahr Perthometer, Göttingen, Germany). For determination of enamel loss, four profiles were recorded across the protected and eroded surfaces. One line was recorded on the protected surface only and served as baseline control for determination of enamel loss. The profile scans were performed in the centre of each specimen at intervals about 250 µm. Control and eroded areas scans were superimposed and the average depth of the area under curve in the eroded area was calculated with specially designed software. The results of the four scans were averaged for each specimen.

Statistical analysis

The software GraphPad Prism 4 version 4.0 for Windows, Graph Pad Software (San Diego, CA, USA) was used. The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Since the assumptions were satisfied, two-way repeated measures ANOVA and Bonferroni *post hoc* test were used. The significance level was set at 5%.

Results

Two-way repeated-measures ANOVA revealed a significant difference between the conditions ($F=17.48$, $p=0.0003$), but not among the dentifrices ($F=1.099$, $p=0.3476$). The interaction between the criteria was not significant ($F=0.0446$, $p=0.957$). Table 1 shows that for the condition erosion plus abrasion, the enamel wear was significantly higher when compared to the condition erosion only ($p<0.05$). There were no significantly differences of the enamel wear between the fluoridated dentifrices and these did not differ from the placebo dentifrice ($p>0.05$).

Discussion

In order to evaluate if the effectiveness of fluoridated dentifrices can be enhanced by increasing their fluoride concentration, this *in situ*/*ex vivo* model tested the effect of a high-concentrated fluoridated dentifrice (5,000 ppm F) on enamel wear. The *in situ*/*ex vivo* protocol was chosen to simulate the *in vivo* situation as closely as possible. This model allowed the formation of an acquired salivary pellicle which might play an important role during the erosive challenge and influence the interaction between fluoride and mineral[20,21]. However, the results of the current *in situ* investigation have to be interpreted within the frame of the study design. It might be assumed that the tongue have an abrasive effect on the palatal located samples. However, Gregg et al.[22] showed that licking of enamel samples had only a minor abrasive effect on eroded enamel. Even though, the volunteers were advised to avoid licking or touching of the enamel blocks, in order to minimize abrasion of the samples. The erosive and abrasive episodes were performed extraorally and were not counterbalanced by saliva properties, such as buffering capacity and salivary flow rate[23,24], which might reduce the demineralisation and enhance the rehardening of the eroded surfaces[7,24]. Moreover, it has to be taken into consideration that the toothpaste slurry was prepared by dilution with water instead of saliva. This design was

conducted, in order to eliminate the influence of minerals present in saliva and focus on the effect of fluoride concentrations in the toothpastes only.

Bovine enamel has been widely used in dental research as a model for human enamel. Even so, bovine enamel was shown to exhibit a higher susceptibility to erosion and abrasion when compared to human enamel[7]. Moreover, polishing of the slabs might also affect the results, as polished surfaces were shown to be more susceptible to acids than natural surfaces[25]. However, for exact profilometric wear analysis, a plan surface polishing is necessary.

The data showed that erosion plus abrasion condition resulted in a greater wear when compared to erosion alone. This finding is consistent with those available in the literature[4,6,7], since the erosive attack causes softening of the enamel, thus leading to an increased susceptibility to abrasive wear[3,4,6,7].

The fluoridated toothpastes (D and C) groups showed a tendency for less enamel wear when compared to the placebo group, in both erosion and erosion plus abrasion situations. However, there was no significant difference among the groups. Although the differences among the toothpastes were not significant, the placebo toothpaste shows a trend for higher enamel loss compared to the fluoridated toothpastes. In addition the study power was below 80% (around 40%), as consequence the results should be interpreted with caution since significant differences between groups might exist but were just not possible to obtain due to the lack of power in the current study. Thus, it is possible to infer that the presence of fluoride in the dentifrice is important, regardless the concentration.

One might have expected to observe at least a better protection from the fluoride dentifrices as demonstrated by previous studies[9,12-14]. The higher amount of fluoride (5,000 ppm F) was not able to protect enamel against erosion and even erosion plus abrasion compared to the 1,100 ppm F dentifrice. At first, it was hypothesized that the differences in pH between the fluoride dentifrices D and C could mask the effects of the higher fluoride concentration of dentifrice D. However, none of the dentifrices used in the present study had a low pH that could enhance the fluoride effect, as was the case of the study by Ganss et al.[9]. Secondly, it may be speculated that the dilution of the dentifrice and the time of fluoride application did not allow the deposit of a calcium fluoride- like layer in an extent enough to prevent subsequent erosive/abrasive attack. On both situations of erosion and erosion plus abrasion, fluoride contacted the enamel for 30 seconds only and afterwards the volunteers washed their mouth with water. This hypothesis could also justify the absence of a significant difference between the high and the regular fluoridated dentifrice.

In the studies by Magalhães et al.[14] and Ganss et al.[9], the pure dentifrices and not the slurries were used, which might have contributed to the better results of the fluoride dentifrices found in these studies. The set-up of the present study probably resulted in a lower fluoride concentration, which enables the adequate deposit of the calcium fluoride-like layer. However, in the clinical situation the dentifrice is always diluted by saliva.

Another factor that could have contributed to the present results is the abrasivity of the toothpastes. However, is has been reported that the abrasivity of the toothpastes is of less relevance for the abrasion of eroded enamel[26]. Additionally, in the present study a short erosive period was used (4 X 1 min) to simulate a clinical condition of frequent drinking of the soft drink. It might be speculated that the fluoride effects might be enhanced when longer erosive periods are applied[9,14]. Further research should evaluate the fluoride response in different periods of acidic challenge.

In summary, it is concluded that under the chosen *in situ*/*ex vivo* conditions, the highly concentrated fluoride dentifrice did not have a significant protective effect on enamel against erosion and erosion plus toothbrush abrasion. However this model should be repeated with a higher number of subjects to increase the study power and confirm the results. Taking this fact into account, risk patients for erosion should benefit from other preventive measures in addition to fluoride dentifrice, since even a highly concentrated fluoride dentifrice is not suitable for enamel erosion prevention.

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References

1. Lussi A. Erosive tooth wear – a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006; 20:1-8.
2. Eisenburger M, Shellis RP, Addy M. Comparative study of wear of enamel induced by alternating and simultaneous combinations of abrasion and erosion *in vitro*. Caries Res 2003; 37:450-5.
3. Davis WB, Winter PJ. The effect of abrasion on enamel and dentine after exposure to dietary acid. Br Dent J 1980; 148:253-6.
4. Jaeggi T, Lussi A. Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an *in situ* study. Caries Res 1999; 33:455-61.
5. Eisenburger M, Hughes J, West NX, Jandt KD, Addy M. Ultrasonication as a method to study enamel demineralization during acid erosion. Caries Res 2000; 34:289-94.
6. Attin T, Knofel S, Buchalla W, Tutuncu R. *In situ* evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res 2001; 35:216-22.
7. Rios D, Honório HM, Magalhães AC, Delbem ACB, Machado MAAM, Silva SMB, Buzalaf MAR. Effect of salivary stimulation on erosion of human and bovine enamel subjected or not to subsequent abrasion: an *in situ*/*ex vivo* study. Caries Res 2006; 40:218-23.
8. Attin T, Deifuss H, Hellwig E. Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999; 33:135-9.
9. Ganss C, Schlueter N, Friedrich D, Klimek J. Efficacy of waiting periods and topical fluoride treatment on toothbrush abrasion of eroded enamel *in situ*. Caries Res 2007; 41:146-51.
10. Ganss C, Klimek J, Brune V, Schurmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine *in situ*. Caries Res 2004; 38:561-6.
11. Vieira A, Lugtenborg M, Ruben JL, Huysmans MCDNJM. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. Caries Res 2006; 40:224-30.
12. Davis WB, Winter PJ. Dietary erosion of adult dentine and enamel. Protection with fluoride toothpaste. Br Dent J 1977; 143:116-9.

13. Bartlett DW, Smith BGN, Wilson RF. Comparison of the effect of fluoride and non-fluoride toothpaste on tooth wear *in vitro* and the influence of enamel fluoride concentration and hardness of enamel. Br Dent J 1994; 176:346-8.
14. Magalhães AC, Rios D, Delbem ACB, Buzalaf MAR, Machado MAAM. Influence of fluoride dentifrice on brushing abrasion of eroded human enamel: an *in situ*/*ex vivo* study. Caries Res 2007; 41:77-9.
15. Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 2006; 40:148-53.
16. Saxegaard E, Rolla G. Fluoride acquisition on and in human enamel during topical application *in vitro*. Scand J Dent Res 1988; 96:523-35.
17. Attin T, Lennon AM, Yakin M, Becker K, Buchalla W, Attin R, Wiegand A. Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site. Clin Oral Investig 2007; 11: 83-8.
18. Barbakow F, Imfeld T, Lutz F, Stookey G, Schemehorm B. Dentin abrasion (RDA), enamel abrasion (REA) and polishing scores of dentifrices sold in Switzerland. Schweiz Monatssch Zahnmed 1989; 99:408-13.
19. Rice DE, Dhabhan DJ, White DJ. Laboratory stain removal and abrasion characteristics of a dentifrice based upon a novel silica technology. J Clin Dent 2001; 12:34-7.
20. Amaechi BT, Higham SM, Edgar WM, Milosevic A. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. J Dent Res 1999; 78:1821-8.
21. Hara AT, Lussi A, Zero DT. Biological Factors. Monogr Oral Sci 2006; 20:88-99.
22. Gregg T, Mace S, West NX, Addy M. A study in vitro of the abrasive effect of the tongue on enamel and dentine softened by acid erosion. Caries Res 2004; 38:557–60.
23. Millward A, Shaw L, Harrington E, Smith AJ. Continuous monitoring of salivary flow rate and pH at the surface of the dentition following consumption of acidic beverages. Caries Res 1997; 31:44-9.
24. Hall AF, Buchanan CA, Millett DT, Creanor SL, Strang R, Foye RH. The effect of saliva on enamel and dentine erosion. J Dent 1999; 27:333-9.

25. Ganss C, Klimek J, Schwarz N. A comparative profilometric *in vitro* study of the susceptibility of polished and natural human enamel and dentine surfaces to erosive demineralization. Arch Oral Biol 2000; 45:897-902.
26. Hooper S, West NK, Pickles MJ, Joinen A, Newcomb RG, Addy M. Investigation of erosion and abrasion on enamel and dentine: a model *in situ* using toothpastes of different abrasivity. J Clin Periodontol 2003; 30:802-8.

Table 1. Mean (±se) wear (µm) of enamel slabs subjected to erosion or erosion + abrasion in the presence of different dentifrices.

Dentifrice	Condition	
	ERO[A]	**ERO + ABR[B]**
Placebo (P)[a]	4.25±0.44	5.09±0.36
1,100 ppm F (C)[a]	3.70±0.36	4.40±0.45
5,000 ppm F (D)[a]	3.45±0.37	4.26±0.57

Distinct lower case and upper case superscripts indicate significant differences among the dentifrice groups and between the experimental conditions, respectively ($p<0.05$).